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CATECHOLAMINE-THYROID INTERRELATIONSHIPS IN RATS AND

GROUND SQUIRRELS AT VARIOUS TEMPERATURES

by

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INTRODUCTION

The ability of an animal to survive in changing environmental situations depends largely upon its ability to maintain homeostasis. Many systems interact in the maintenance of homeostasis, and the involvement of the endocrine system is well documented. The complexity of the interaction of the endocrine system is emphasized with each new investigation, and is seen in the interrelationships of the thyroid and adrenal gland during cold exposure of animals.

The thyroid has been shown to increase its secretory activity when an animal is exposed to low temperatures. The result is thought to be important in increasing the heat production of the animal.

The adrenal hormones, particularly the catecholamines, have also been shown to be associated with the survival of the animal in the cold.

Because both the catecholamines and the thyroid hormones participate in the adjustment to cold exposure there have been attempts made to establish a link or a synergistic relationship between the adrenal and the thyroid glands. The present study was undertaken to investigate the effect of noradrenaline on the uptake of I^{131} and its subsequent distribution in the thyroid hormones of animals exposed to various temperatures. The experiments were designed to compare the response of the thyroid to noradrenaline stimulation in the rat, with the ground squirrel (*Spermophilus tridecemlineatus*).

REVIEW OF THE LITERATURE

Studies of the intriguing increases in both thyroid and adrenal activity following cold exposure has produced an abundance of relevant data. This area has been investigated for almost 100 years. However, much uncertainty persists as to the basic physiological activity of the thyroid and adrenal glands during cold exposure.

It is generally agreed that thyroid hormones are involved in the maintenance of the animal in cold conditions. It has been shown that increased metabolic responses are associated with increased thyroid activity in homeotherms and summer hibernators exposed to cold (Hoffman, 1964). The requirement of the thyroid hormones for survival in the cold has been documented in many investigations and reviews (Carlson, 1958; Pitt-Rivers and Tata, 1959; Deane and Lyman, 1954; LeBlond and Gross, 1943; LeBlond et al., 1944; Cramer, 1928; Rand et al., 1952; Hoch, 1962; Solomon and Dowling, 1960; Sellers and You, 1950). There is disagreement, however, as to the degree of involvement of the thyroid gland in temperature regulation. For example, Heroux (1967) has shown that increased thyroid activity induced by cold exposure of rats is secondary to increased fecal loss of iodine. The result was an increase in thyroid hormone synthesis which led to the normally accepted picture of increased thyroid activity. The multiplicity of actions of the thyroid hormones may be necessary for survival, but thyroid hormones may not play a causative role in the establishment of the heat production mechanism such as non-shivering thermogenesis (Heroux and Brauer, 1965).

The importance of noradrenaline in cold adaptation has been shown in many investigations. Cold acclimation has been shown to be accompanied by increased sensitivity to the metabolic actions of noradrenaline (Depocas, 1960; Hsieh et al.,1957). During cold exposure it has been demonstrated that there was an increase in noradrenaline excretion associated with an increase in the calorigenic action of noradrenaline such as increased myocardial activity (Cottle and Carlson, 1956; LeBlanc and Pouliot, 1964).

Increased sensitivity of sympathectomized animals to extreme environment temperatures has been noted (Ramey and Goldstein, 1957). Maickel et al. (1967) have shown that the sympathetic nervous system is essential for existence at low temperatures.

The phenomena of cold adaptation may involve thyroid and adrenal interaction. Indeed, it has been postulated that the main function of thyroxine in cold acclimation is potentiation of catecholamines (Swanson, 1956, and 1957; Trendelenburg, 1953; Harrison, 1964; Money, 1955). The thyroid hormones are thought to regulate the magnitude of the calorigenic and hyperglycemic action of catecholamines (Ellis, 1956). It has been shown that increased amounts of thyroxine potentiate while decreased amounts decrease the calorigenic action of catecholamines (Hoch, 1962). This may mean that the catecholamines in these instances are the primary agents controlling cellular increases in the calorigenic action and that thyroxine potentiates the catecholamines. In addition, thyroxine has been reported to potentiate the actions of the adrenal medullary hormones by inhibiting the enzymes which inactivate the catecholamines. However, one can hardly find a more conflicting series of reports than those on the effects of thyroxine on monoamine oxidase and catechol-O-methyl transferase.

Thyroxine has been shown to increase monoamine oxidase activity in whole-cell homogenates (Klein, 1939). Wurtman et al. (1963) reported that there was a decrease in monoamine oxidase activity in the myocardium in rats fed thyroid tissue. Zile (1960) showed that there was no change in myocardial and central nervous system monoamine oxidase levels in animals whose hepatic monoamine oxidase content decreased by 59 percent. D'Ioria and Leduc (1960) showed that in thyroid fed rats the catechol-O-methyl transferase activity was decreased. Wurtman et al. (1963) found no change in the transferase activity in rat myocardium.

If the potentiation of the catecholamines by thyroxine is a reality, its action does not appear to be a uniform inhibition of the inactivation mechanisms of catecholamines.

There is an opinion that adrenaline will stimulate the release of thyroid hormones. However, in a recent report (Ahn et al., 1969) it was shown that adrenaline decreased the blood flow to the thyroid of dogs with no change in the secretion rate of thyroid hormones. This was similar to work of Soderberg (1958) who showed that, in rabbits and cats, adrenaline decreased the blood flow to the thyroid and had little or no effect on the absolute amount of radioactivity released from the thyroid. Ackerman and Aron (1958) found an increase in the venous effluent of dogs following intravenous adrenaline infusion. There is no evidence that there is a striking change in the thyroid secretion rate upon catecholamine administration.

The effect of the catecholamines on the chain of events involved in the synthesis of thyroid hormones has not been studied in detail. Several reports have described inhibition (Soffer _et al_., 1949; Williams _et al_., 1949) and augmentation (Botkin and Tew, 1952; Reiss _et al_., 1949) of thyroidal I^{131} uptake after adrenaline infusion. No attempt has been made to demonstrate a change in thyroid synthesis pattern with regard to changes in the ratio of the chromatographic fractions of thyroid components during catecholamine stimulation.

To summarize, there is a suggestion that catecholamines potentiate the thyroid hormones and that the thyroid hormones, in turn, are important in the actions of the catecholamines. However, little evidence has been presented to substantiate either possibility. More work is needed to clarify the relationship between activity of the thyroid and the catecholamines. This is especially true of the interactions that may exist during thermal acclimatization.

MATERIALS AND METHODS

Males and females Sprague-Dawley-Rolfsmeyer rats were housed at 25°C±0.5. Illumination was maintained on an 8 A.M.-10 P.M. schedule. Wayne Lab Blox and water were provided *ad libitum*. Ground squirrels (*Spermophilus tridecemlineatus*) were maintained under similar conditions.

Four experimental groups consisting of six rats and six ground squirrels, of mixed sexes, were selected for each experiment. The experimental groups were:

a. Animals that received a 1 ml injection containing 4 µc of I^{131} (Abbott Laboratories, Sodium Iodide carrier free). Injections were given I.P. (intraperitoneal).

b. Animals that received a 1 ml injection containing 4 µc of I^{131} and a second injection I.P. of 0.4 mg of tapazole (methimazole)/ 100 gms body weight. The second injection was given 24 hours after the I^{131} injection. (Tapazole was obtained from the Department of Animal Science, Iowa State University.)

c. Animals that received the same I^{131} and tapazole treatment as the above group, plus an injection of 0.2 mg noradrenaline I.P. 24 hours post tapazole treatment. (Noradrenaline, Nutritional Biochemicals, Control No. 8829.)

d. Animals that received similar I^{131} and tapazole treatment as the above groups, plus an injection of 0.5 mg hexamethonium chloride I.P. (Nutritional Biochemicals, Control No. 8899.)

The experimental groups were exposed to either 25°C, 10°C, or 5°C. All temperatures were controlled within ± 0.5°C. The experimental groups were maintained at the stated temperatures for either 4, 8, or 12 hours. The animals were then removed from the environmental chamber and killed with nembutal.

A blood sample was taken by direct heart puncture. The radioactivity present in the blood was measured as a percentage of the activity remaining in a 1 ml sample of the injected I^{131} stock solution. The blood radioactivity was recorded as a percent per ml.

The thyroids were removed, weighed and placed in iced Kreb's solution. The total thyroid radioactivity was measured as a percentage of the activity remaining in 1 ml of stock solution and recorded as a percent per mg thyroid tissue. The thyroids were then homogenized and incubated with pancreatin (Pancreatin 5X N.F., Nutritional Biochemicals) according to Inoue (1966). A 20 µl aliquot of each digest was transferred to a thin layer chromatography (TLC) plate for chromatographic separation and development according to Shapiro and Gordon (1966). The chromatographic fractions - origin (O), iodine (I^-), monoiodotyrosine (MIT), diiodotyrosine (DIT),3,5,3' triiodothyronine (T_3), and thyroxine (T_4) - were removed from the TLC plate by scraping each fraction into a test tube. The radioactivity of each fraction was recorded as a percentage of the radioactivity in a 20 ul sample of the digest.

A Packard Auto-Gamm Spectrometer was used to record all radioactivity.

A statistical analysis of variance was conducted by the Iowa State University Statistical Laboratory. The effects of the interaction for species, treatment, temperature, and time exposure were tested for significance.

RESULTS

Analysis of Variance

Analysis of variance for radioactivity of the blood is given in Table 1. The temperature effect (T), treatment effect (Tr), and species effect (S) were shown to be highly significant at the 1% level. Also, the interaction of the species-temperature (ST), species-treatment (STr), temperature-treatment (TTr), and species-time-treatment (STiTr) were significant at the 1% level.

Analysis of variance for total thyroid activity is given in Table 2. Significance at the 1% level was shown for the species, temperature, and treatment effects. Interactions of the species-temperature, and species-treatment effects were significant at the 1% level while the temperature-treatment interaction was significant at the 5% level.

Tables 3-5 show the analysis of variance for the radioactivity in the fractions of the thyroid chromatograph. The results show that the treatment effect was highly significant in all chromatographic fractions. The time-treatment interaction was significant at the 1% level in all fractions except the MIT fraction which was significant at the 5% level. Temperature had a highly significant effect on the radioactivity located in the origin, I^-, and T_3 fractions. The time effect was highly significant in influencing the activity located in the origin, MIT, T_3, and T_4 fractions, while the species effect was significant only in the origin fraction. The results showed that the species-temperature interactions, were significant in all fractions excluding the DIT fraction.

Total Radioactivity in the Thyroid

Comparison of radioactivity in the thyroid of rats exposed to 25°C (Figure 1) with animals exposed to 5°C (Figure 2) showed that noradrenaline treatment had an opposite effect at the two temperatures. At 25°C noradrenaline caused a decrease in the radioactivity in the thyroid, and an increase at 5°C. Animals that received the single injection of I^{131} showed a slight increase in activity at 25°C, but a sharp decrease in activity at 5°C. Hexamethonium treatment at 25°C produced a decrease in the activity similar to that of noradrenaline; however, at 5°C hexamethonium caused a decrease in the radioactivity. Tapazole treatment at both temperatures showed a slight decrease in thyroid radioactivity. It can be seen (Figures 1 and 2), that the radioactivity levels, regardless of the treatment, are generally higher at 25°C than at 5°C.

The treatment effects on the ground squirrel thyroid radioactivity are shown in Figures 3 and 4. It can be seen that noradrenaline produced results comparable to the rat (Figures 1 and 2). There was a decrease in activity at 25°C and an increase at 5°C. The I^{131} treatment showed an increase at 25°C and a decrease at 5°C. Hexamethonium treatment also produced comparable results with those of the rat. The treatment produced a decrease in activity at both temperatures, but it can be seen that hexamethonium did not lower the total radioactivity in the ground squirrel thyroid to the same extent as in the rat thyroid. Tapazole treatment produced, at both temperatures, a decrease in the thyroid radioactivity of the ground squirrel.

Chromatographic Analysis

Rats exposed to 25^{o}C and treated with noradrenaline and hexamethonium showed (Table 9) a progressive decrease in the radioactivity of the T_3 and T_4 fractions. Hexamethonium-treated animals also showed a decrease in the I^- and DIT fractions and an increased radioactivity in the MIT fraction.

Animals receiving only I^{131} showed a slight increase in the activity in all fractions. The tapazole-treated animals did not show a definite trend in any of the chromatographic fractions.

Data for rats exposed to 5^{o}C (Table 10) and treated with noradrenaline showed a slight increased activity in all chromatographic fractions. The hexamethonium-treated rats showed a decrease in the radioactivity located in the I^-, DIT, T_3, and T_4 fractions, and an increase in the MIT fraction. The increase in the MIT fraction was also found in the 25^{o}C exposed rats. Animals treated with only I^{131} showed a decrease in all fractions except the MIT fraction, which showed an increase. The tapazole-treated animals did not show any change in the radioactivity of the fractions.

Data obtained with the ground squirrels exposed to 25^{o}C and 5^{o}C (Tables 11 and 12) indicated that, at the same temperature, the results were similar to those for the rats. At 25^{o}C there was a decrease in the activity in the DIT, T_3, and T_4 fractions in animals treated with noradrenaline and hexamethonium. Animals exposed to 5^{o}C and treated with noradrenaline showed an increase in the I^-, MIT, DIT, and T_3 fractions. At both 25^{o}C and 5^{o}C the MIT fraction increased in activity in animals treated with hexamethonium. This was similar to results obtained for the rats exposed to 25°C and 5°C.

The results for the 10°C exposure of rats and ground squirrels were intermediate to those obtained at 25°C and 5°C and are presented only in the analysis of variance (Tables 1 - 8).

Standard Deviations for Thyroid Radioactivity

The treatment effects on the rat and ground squirrel thyroid radioactivity, expressed as the mean percentage of radioactivity per mg thyroid, are given in Tables 13-14. The standard deviations of the means are also presented in Tables 13-14.

DISCUSSION

Effects of Cold on Thyroidal I^{131}

It has been shown that cold exposure produced an increased synthesis of thyroid hormones as measured by the incorporation of I^{131} into the circulating hormones (Brown-Grant, 1956). In experiments described here (Tables 10 and 12), a progressive decrease in radioactivity was shown in the DIT, T_3 and T_4 fractions of the thyroid homogenates (Tables 10 - 12) and in the total thyroid activity for both rats and ground squirrels exposed to 5°C (Figures 2 and 4). If one interprets the decreased radioactivity found in both the total thyroid and thyroid T_3 and T_4 fractions as an indication of hormone release, then the results are in accord with those reported in the literature (Hart, 1960; Smith and Hoijer, 1962). It was noted that thyroid radioactivity was lower in the ground squirrels than in the rats at both 25°C and 5°C (Figures 1 - 4). Hoffman and Zarrow (1958) suggested that the sensitivity of the TSH releasing system in the ground squirrel was decreased during the winter months even for those animals maintained at 22 - 25°C. The data for the ground squirrels in this investigation were obtained in the months of December-February, and could account for the lowering of the uptake of radioactive iodide in the ground squirrel's thyroid in comparison with the rats (Figures 1 - 4).

Catecholamines and Thyroid Radioactivity

Administration of adrenaline to animals results in hyperplasia and hypertrophy of the thyroid (Goetsch, 1940; Money, 1955). Ackerman and Arons (1958) reported that intravenously administered adrenaline or noradrenaline to dogs increased the rate of secretion of the thyroid hormones; however, Ahn et al. (1969) reported that adrenaline decreased the blood flow to the thyroids in dogs, but did not change the secretion rate for the thyroid hormones.

Data from the author's experiments indicate that noradrenaline caused a significant decrease (Tables 13-14) in the total thyroid radioactivity, when compared with the tapazole treated animals, in both the rat and ground squirrel exposed to 25°C (Figures 1 and 3). Comparisons of the chromatographic fractions of rats and ground squirrels exposed to 25°C and treated with noradrenaline (Tables 9 and 11) reflect a decrease in radioactivity in the T_3 and T_4 fractions for both animals. One could conclude that noradrenaline increased thyroid hormone release at 25°C.

Data from the 5°C experiments are more difficult to interpret. Both rats and ground squirrels showed a significant increase (Tables 13-14) in total thyroid radioactivity following noradrenaline administration (Figures 2 and 4). No pattern of fluctuations occurred in the location of the radioactivity in the chromatographic analysis of the homogenates (Tables 10 and 12). Because of the experimental design, the total radioactivity in the homogenate could increase or decrease, and if the radioactivity in all chromatographic fractions remained proportionally constant, a change in iodide uptake, synthesis and release of thyroid hormones would be difficult to determine. The author suggests that at 5°C noradrenaline caused an increased iodide uptake, which was reflected by the increased total thyroid radioactivity (Figures 2 and 4). Also, noradrenaline increased the synthesis and release of the thyroid hormones. If one correlates the increase in total thyroid radioactivity with the constant proportionality seen in the distribution of radioactivity in the thyroid fractions (Tables 10 and 12), the increased radioactive uptake was probably passed along to all the fractions, and indicated a synthesis of the thyroid hormones. No increase occurred in the end product which suggested an increased thyroid release. It is obvious that the above is speculative and more data are needed to substantiate the suggestion.

Hexamethonium Treatment and the Thyroid

The involvement of the sympathetic system in the control of cellular metabolism during cold exposure was demonstrated by Hsieh et al. (1957). They showed that noradrenaline was important in increasing the oxygen consumption in cold exposed animals. They also demonstrated that hexamethonium blocked increased oxygen consumption if given before cold exposure and greatly reduced oxygen consumption if given after the animal was placed in a cold environment. Hexamethonium is a preganglionic blocking agent (Wurtman, 1966), and decreases activity of the sympathetic nervous system. This investigation showed that hexamethonium decreased the total thyroid radioactivity at both 25°C and 5°C in the rat and ground squirrel. Chromatographic analysis of the thyroids for both animals indicated a general decrease in all the fractions except the MIT fraction which showed an increase in radioactivity (Tables 9 - 12). The thyroid vascular supply is great and probably more blood flows through the thyroid in relationship to its size than through most organs (Turner, 1964). If hexamethonium decreased the flow of blood to the thyroid, a general decrease in thyroid radioactivity would occur. The increase in the MIT fraction at this time can not be explained.

Tapazole and Thyroid Radioactivity

Tapazole (1-methyl-2-mercaptoimidiazole) is a thiocarbonamide compound that has been found to have goitrogenic activity. It has been suggested that tapazole inhibits the peroxidase system that is necessary for oxidation of trapped iodide and incorporation into tyrosine (Solomon and Dowling, 1960).

Tapazole treatment decreased the total thyroid radioactivity in both the rat and ground squirrel at $25^{o}C$ and $5^{o}C$ (Figures 1 - 4). Inspection of the chromatographic data (Tables 9-12) indicated that there was a slight decrease of radioactivity in the T_3 and T_4 fractions at both temperatures. Tapazole has been reported to have great effect in blocking uptake of I^{131} (Hiller and Strauss, 1954). A review of the literature did not reveal the half-life of tapazole but the treatment levels normally given to man and other animals indicate that the half-life is probably less than one day. This would explain the presence of the radioactivity in all fractions of the chromatograph (Tables 9 - 12). The animals were not exposed to the cold for 24 hours following the tapazole treatment. The effectiveness of the agent was probably greatly reduced and the thyroid converted the trapped I^{131} into the thyroid hormones. The decrease in the total thyroid radioactivity was probably due to the normal tonic release of thyroid hormones.

Blood Analysis

Correlation of the radioactivity levels in the blood with specific events in the thyroid was not possible. The changes in the blood radioactivity concentration did not follow a consistent pattern when correlated with uptake or release of radioactivity from the thyroid. The analysis of variance (Table 1) for blood suggested that the treatment effects had a significant influence on the blood radioactivity levels, but this may be an example where statistical significance does not imply biological significance.

CONCLUSIONS

The rat and ground squirrel responded in a similar manner to the effects of cold, hexamethonium, tapazole, and noradrenaline. Exposure to the cold elicited an increase in the release of thyroid hormones. The ground squirrels showed less uptake of radioactivity into the thyroids than did the rats. A decreased sensitivity of the TSH releasing mechanisms in the ground squirrel could account for the disparity between the percentage uptake of radioactivity in rat and that in ground squirrel.

Hexamethonium caused a decrease in the thyroid radioactivity at both 25°C and 5°C. The decrease was possibly the result of a decreased blood flow to the thyroid (Wurtman, 1966). Tapazole resulted in a decrease of radioactivity in the thyroid, but the decrease was less pronounced at 25°C than at 5°C (Figures 1 - 4). It is possible that the decrease in activity was the result of the temperature-treatment interaction and not due primarily to the drug effect. At 25°C noradrenaline caused a decrease in the thyroid radioactivity which was probably due to the increased release of the thyroid hormones. At 5°C noradrenaline elicited an increase in the total radioactivity in the thyroid gland. While it is quite conjectural, it is possible that the increase in radioactivity reflects an increased activity of the thyroid in the processes of uptake, synthesis and release of hormones. This suggestion is not without basis because it has been suggested that the catecholamines increase the release of TSH (Money, 1955). TSH has been shown to increase all phases of thyroid activity (Turner, 1964).

SUMMARY

1. Sprague-Dawley-Rolfsmeyer rats and ground squirrels (<u>Spermophilus tridecemlineatus</u>) were injected with 4 μc of I^{131}, and divided into four groups of 6 rats and 6 ground squirrels which received the following treatments:
   a. Those animals that received no further injections.
   b. Those animals that received 0.4 mg tapazole (I.P.).
   c. Those animals that received the tapazole treatment plus an injection of hexamethonium (0.5 mg).
   d. Those animals that received the tapazole treatment plus 0.2 mg noradrenaline.
2. Each animal group was exposed to 25°C for 4, 8, or 12 hours and 4 duplicate groups of animals were exposed to 5°C for either 4, 8, or 12 hours.
3. The effect of the treatment, temperature and duration of exposure was examined with respect to the concentration of radioactivity located in the total thyroid and in the TLC fractions of the thyroid homogenates.
4. A statistical analysis of variance for the interactions of the species, temperature, time and treatment effects was conducted.
5. The total thyroid radioactivity values disclosed:
   a. That decreased temperature resulted in a release of radioactivity from the thyroid.
   b. That tapazole, hexamethonium and noradrenaline reduced the radioactivity in the thyroid at 25°C over the duration of exposure.

c. Noradrenaline caused an increase in the thyroid radioactivity at $5^{o}C$.

d. Hexamethonium and tapazole caused a decrease in the total thyroid radioactivity, in animals exposed to $5^{o}C$.

6. The chromatographic values disclosed:

a. That noradrenaline at $25^{o}C$ caused a decrease in the T_3 and T_4 fractions.

b. Noradrenaline at $5^{o}C$ had no apparent effect on the specific location of the radioactivity in the various chromatographic fractions.

c. Hexamethonium and tapazole showed no effect on the distribution of the radioactivity in the chromatographic fractions except for an increase in the MIT fraction following hexamethonium treatment.

7. It is concluded that:

a. Cold exposure increased the release of thyroid hormones from the thyroid.

b. Noradrenaline treatment at $25^{o}C$ increased the release of thyroid hormones. At $5^{o}C$ noradrenaline increased the total activity of the thyroid.

c. The action of hexamethonium on thyroid activity was possibly the indirect affect of lowered sympathetic activity and decreased blood flow.

d. Tapazole treatment resulted in a decrease in the thyroid radioactivity that might have been the result of a tonic release of hormones at 25°C and not due to the treatment. It is possible that the interaction of temperature and treatment influenced the decrease in radioactivity at 5°C.

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APPENDIX

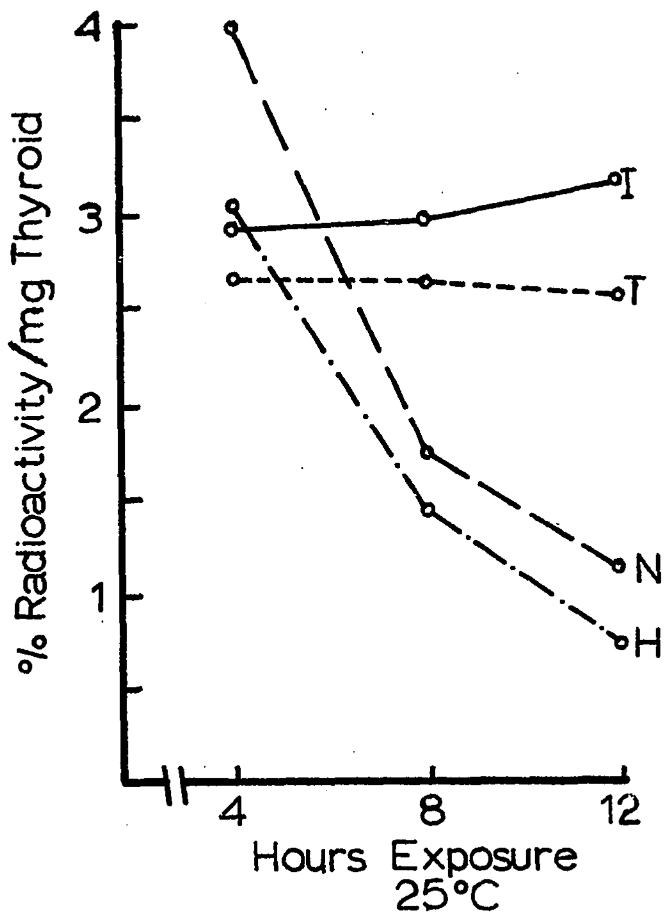

Figure 1. Treatment effects on rats exposed to 25°C. The abscissa is exposure time in hours. The ordinate is the percentage of injected radioactivity, located in the thyroid and expressed as a percent per mg thyroid tissue.

I = I^{131}
T = Tapazole
N = Noradrenaline
H = Hexamethonium

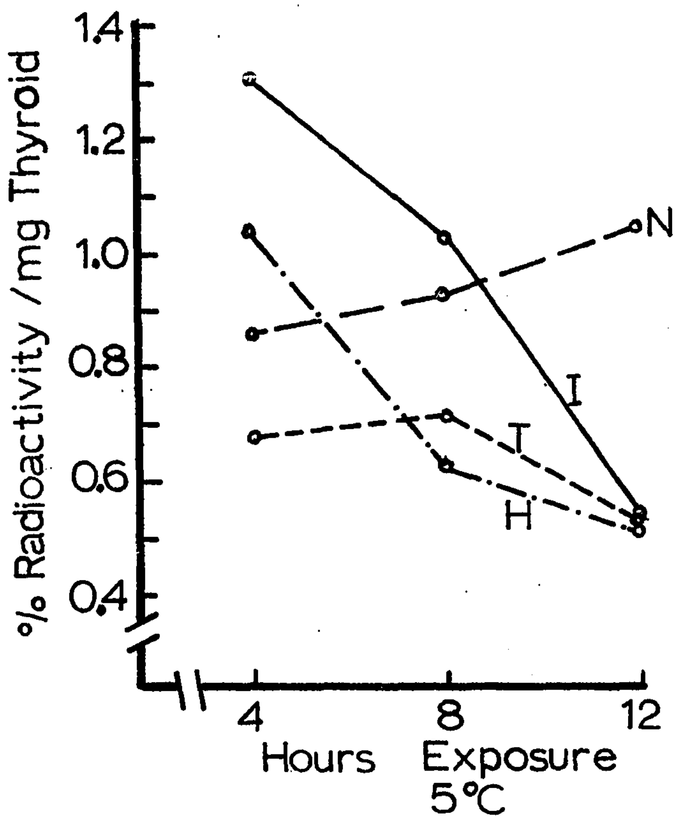

Figure 2. Treatment effects on rats exposed to 5°C. The abscissa is exposure time in hours. The ordinate is the percentage of injected radioactivity located in the thyroid and expressed as a percent per mg thyroid tissue.

I = I^{131}
T = Tapazole
N = Noradrenaline
H = Hexamethonium

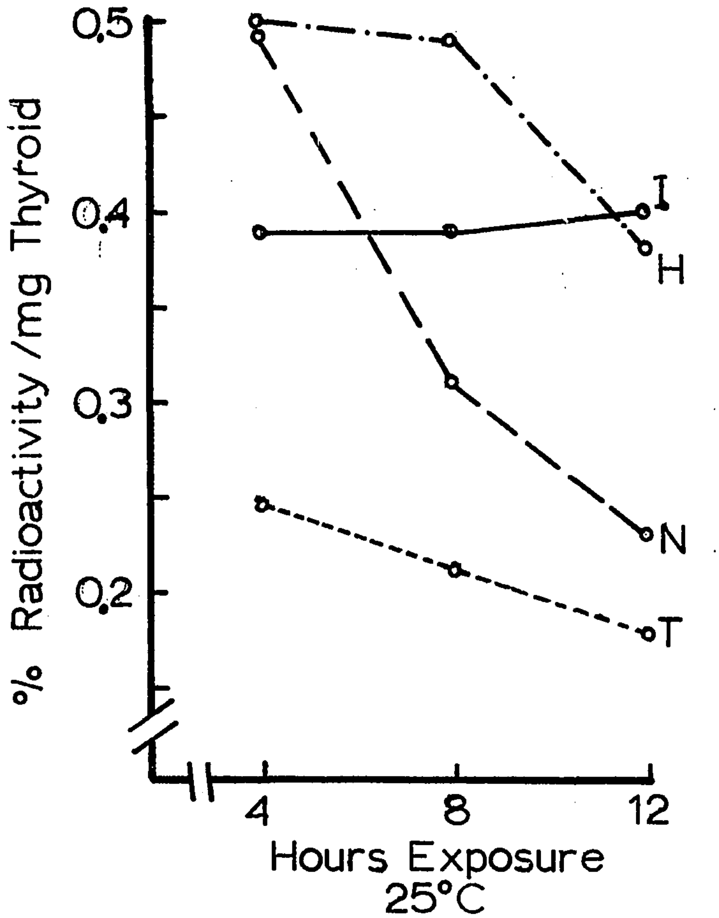

Figure 3. Treatment effects on ground squirrels exposed to 25°C. The abscissa is exposure time in hours. The ordinate is the percentage of injected radioactivity located in the thyroid as expressed as a percent per mg thyroid tissue.

I = I^{131}
T = Tapazole
N = Noradrenaline
H = Hexamethonium

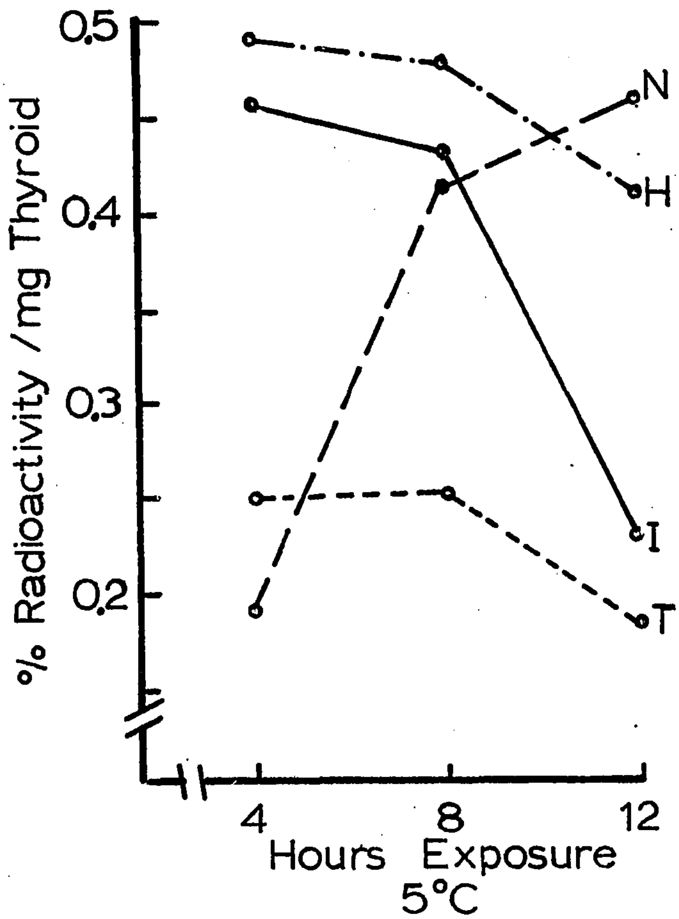

Figure 4. Treatment effects on ground squirrels exposed to 5°C. The abscissa is exposure time in hours. The ordinate is the percentage of radioactivity located in the thyroid and expressed as a percent per mg thyroid tissue.

I = I^{131}
T = Tapazole
N = Noradrenaline
H = Hexamethonium

Table 1. Analysis of variance for radioactivity in blood[a]

Due to	Sum of squares	Degree of freedom	Mean square	F
Species effect (S)	0.00390457	1	0.0039051	2264.385**
Temperature effect (T)	0.0030827	2	0.0015413	893.600**
ST	0.0027545	2	0.0013772	798.473**
Time effect (Ti)	0.0000048	2	0.0000024	1.400
STi	0.0000005	2	0.0000002	0.145
TTi	0.0000047	4	0.0000011	0.689
STTi	0.0000033	4	0.0000008	0.484
Treatment effect (TR)	0.0001324	3	0.0000441	25.588**
STr	0.0000269	3	0.0000089	5.209**
TTr	0.0002448	6	0.0000408	23.661**
TiTr	0.0000113	6	0.0000018	1.094
STiTr	0.0001845	6	0.0000307	17.833**
TTiTr	0.0000443	12	0.0000036	2.141
Error	0.0000310	18	0.0000017	

[a]Radioactivity per ml blood.

** Highly significant at the 1% level (here and throughout).

Table 2. Analysis of variance for radioactivity in thyroid[a]

Due to	Sum of squares	Degree of freedom	Mean square	F
S	23.486	1	23.486	173.946**
T	9.615	2	4.807	35.608**
ST	8.549	2	4.274	31.660**
Ti	0.617	2	0.308	2.285
STi	0.374	2	0.187	1.385
TTi	1.655	4	0.413	3.065*
STTi	1.374	4	0.343	2.545
Tr	2.492	3	0.830	6.152**
STr	2.528	3	0.842	6.243**
TTr	1.3111	6	0.218	1.618
TiTr	1.494	6	0.249	1.844
STTr	1.260	6	0.210	1.555
TTiTr	2.442	12	0.203	1.507
Error	2.430	18	0.135	

[a]Radioactivity per mg thyroid tissue.

*Significant at 5% level (here and throughout).

Table 3. Analysis of variance for radioactivity in origin fraction of thin-layer chromatography of thyroid homogenate

Due to	Sum of squares	Degree of freedom	Mean square	F
S	0.605	1	0.605	28.348**
T	0.292	2	0.146	6.852**
ST	0.405	2	0.202	9.508**
Ti	0.303	2	0.151	7.106**
ST	0.083	2	0.041	1.952
TTi	0.279	4	0.069	3.270
STTi	0.305	4	0.076	3.582
Tr	1.295	3	0.431	20.226**
STr	0.112	3	0.037	1.761
TTr	0.714	6	0.119	5.577**
TiTr	0.679	6	0.113	5.310**
STTr	0.303	6	0.050	2.366
TTiTr	0.650	12	0.054	2.541*
Error	0.384	18	0.021	

Table 4. Analysis of variance for radioactivity in I^- fraction of thin-layer chromatography of thyroid homogenate

Due to	Sum of squares	Degree of freedom	Mean square	F
S	0.003	1	0.003	0.037
T	2.177	2	1.088	11.643**
ST	0.833	2	0.416	4.457**
Ti	0.163	2	0.081	0.873
STi	0.274	2	0.137	1.467
Ti	1.316	4	0.329	3.520
STTi	0.462	4	0.115	1.235
Tr	4.859	3	1.619	17.321**
STr	0.163	3	0.054	0.583
TTr	3.550	6	0.591	6.327**
TiTr	6.881	6	1.146	12.264**
STTr	1.334	6	0.222	2.377
TTiTr	2.865	12	0.238	2.553
Error	1.683	18	0.093	

Table 5. Analysis of variance for radioactivity in MIT fraction of thin-layer chromatography of thyroid homogenate

Due to	Sum of squares	Degree of freedom	Mean square	F
S	0.420	1	0.420	0.052
T	40.185	2	20.092	2.491
ST	71.333	2	35.666	4.423*
Ti	111.764	2	55.882	6.930**
ST	6.121	2	3.060	0.379
TTi	19.485	4	4.871	0.604
STTi	33.513	4	8.378	1.039
Tr	372.779	3	124.259	15.409**
STr	3.719	3	1.239	0.153
TTr	171.803	6	28.633	3.550*
TiTr	257.659	6	42.943	5.325**
STTr	204.521	6	34.086	4.227**
TTiTr	79.591	12	6.632	0.822
Error	145.147	18	8.063	

Table 6. Analysis of variance for radioactivity in DIT fraction of thin-layer chromatography of thyroid homogenate

Due to	Sum of square	Degree of freedom	Mean square	F
S	3.423	1	3.423	0.624
T	7.035	2	3.517	0.641
ST	20.943	2	10.471	1.910
Ti	10.486	2	5.243	0.956
STi	4.153	2	2.076	0.378
TTi	16.753	4	4.188	0.764
STTi	15.412	4	3.853	0.703
Tr	314.102	3	104.700	19.103**
STr	2.661	3	0.887	0.161
TTr	13.770	6	2.295	0.418
TiTr	159.841	6	26.640	4.860**
STTr	80.406	6	13.401	2.445
TTiTr	69.801	12	5.816	1.061
Error	98.655	18	5.480	

Table 7. Analysis of variance for radioactivity in T_4 fraction of thin-layer chromatography of thyroid homogenate

Due to	Sum of squares	Degree of freedom	Mean square	F
S	5.066	1	5.066	1.871
T	0.421	2	0.210	0.077
ST	39.607	2	19.803	7.315**
Ti	107.254	2	53.627	19.809**
STi	8.593	2	4.296	1.587
TTi	2.687	4	0.671	0.248
STTi	28.015	4	7.003	2.587
Tr	56.538	3	18.846	6.961**
STr	5.644	3	1.881	0.695
TTr	88.618	6	14.769	5.455**
TiTr	49.617	6	8.269	3.054*
STTr	34.984	6	5.83	2.153
TTiTr	71.725	12	5.977	2.207
Error	48.730	18	2.707	

Table 8. Analysis of variance for radioactivity in T_3 fraction of thin-layer chromatography of thyroid homogenate

Due to	Sum of squares	Degree of freedom	Mean square	F
S	0.045	1	0.045	0.200
T	1.330	2	0.665	3.843**
ST	3.360	2	1.660	9.709**
Ti	5.340	2	2.670	15.428**
STi	0.272	2	0.136	0.787
TTi	2.078	4	0.519	3.002*
STTi	1.371	4	0.342	1.981
Tr	5.160	3	1.720	9.939**
STr	0.827	3	0.275	1.593
TTr	10.255	6	1.709	9.875**
TiTr	5.575	6	0.929	5.369**
STTr	1.966	6	0.327	1.894
TTiTr	8.038	12	0.669	3.870*
Error	3.115	18	0.173	

Table 9. Mean values of radioactivity in thyroid chromatographic fractions of rat exposed to 25°C[a]

Time (hr)[b]	Treatment	O	I^-	MIT	DIT	T_3	T_4
	I^{131}[c]						
4		3.6	6.3	23.7	39.3	3.2	17.1
8		3.5	5.6	22.4	40.3	3.1	16.5
12		3.7	6.4	23.5	40.2	3.4	18.1
	Nor.[d]						
4		3.5	6.0	23.9	40.2	3.2	17.0
8		3.5	5.9	24.0	42.0	1.9	10.0
12		3.6	6.3	24.9	41.9	0.0	5.4
	Hex.[e]						
4		3.6	6.3	24.0	40.9	3.5	18.0
8		2.8	5.1	45.7	26.9	1.1	12.1
12		2.9	5.7	46.5	26.9	0.8	12.0
	Tap.[f]						
4		3.4	7.0	25.1	38.4	3.1	18.1
8		3.6	6.6	24.1	41.0	3.5	17.9
12		3.5	6.0	24.3	40.9	3.3	16.0

[a]Radioactivity expressed as a percentage of sample.

[b]Hours after exposure to 25°C.

[c]I^{131} injection of radioactivity I^{131} alone.

[d]Nor injection of I^{131} plus tapazole and noradrenaline.

[e]Hex. injection of I^{131} plus tapazole and hexamethanium.

[f]Tap. injection of I^{131} and tapazole. These footnotes apply here and throughout remainder of tables.

Table 10. Mean values of radioactivity in thyroid chromatographic fractions of rats exposed to $5^{o}C^{a}$

Time (hrs)	Treatment	O	I^-	MIT	DIT	T_3	T_4
	I^{131}						
4		3.6	6.5	24.2	41.1	3.0	16.6
8		3.5	6.8	25.1	38.7	2.6	15.9
12		3.3	6.5	25.3	37.4	1.9	13.1
	Nor.						
4		3.5	6.0	23.7	41.7	3.0	16.8
8		3.5	6.8	24.1	41.9	3.6	17.4
12		3.6	7.2	24.7	42.4	3.7	17.4
	Hex.						
4		3.6	6.6	23.8	39.3	3.3	17.1
8		3.2	6.2	25.3	37.4	3.1	16.8
12		3.5	6.0	27.4	36.1	2.9	15.1
	Tap.						
4		3.5	6.4	23.5	41.4	3.0	15.8
8		3.6	6.8	24.5	41.9	2.9	15.4
12		3.4	6.3	23.4	41.3	3.1	15.4

[a]Radioactivity expressed as a percentage of sample. These footnotes apply here and throughout the remainder of the tables.

Table 11. Mean values of radioactivity in thyroid chromatographic fractions in ground squirrels exposed to 25°C

Time (hrs)	Treatment	O	I^-	MIT	DIT	T_3	T_4
	I^{131}						
4		3.6	6.2	24.1	40.4	3.2	17.3
8		3.5	6.0	22.9	40.0	3.4	16.8
12		3.6	6.4	24.0	41.4	3.3	19.1
	Nor.						
4		3.6	6.1	24.1	40.1	3.4	17.0
8		3.6	6.0	24.9	41.6	2.0	15.3
12		3.5	6.2	25.3	40.4	0.5	10.4
	Hex.						
4		3.6	6.3	24.2	41.0	3.5	18.2
8		2.8	6.3	25.3	41.2	3.4	17.1
12		2.6	5.4	35.8	34.2	2.4	15.6
	Tap.						
4		3.4	7.2	25.0	39.1	3.4	17.5
8		4.0	6.5	24.3	41.9	3.6	16.4
12		3.4	6.0	24.4	41.0	3.0	16.0

Table 12. Mean values of radioactivity in thyroid chromatographic fractions in ground squirrels exposed to 5°C

Time (hrs)	Treatment	O	I^-	MIT	DIT	T_3	T_4
	I^{131}						
4		3.7	6.6	24.4	41.2	3.1	16.6
8		3.5	6.3	24.0	41.3	2.7	15.7
12		3.2	6.4	25.4	38.3	2.1	13.1
	Nor.						
4		3.5	6.0	23.9	41.3	3.3	16.7
8		3.4	7.2	25.3	42.2	3.2	15.6
12		2.9	7.3	25.3	43.5	3.5	15.0
	Hex.						
4		3.6	6.5	23.1	42.2	3.4	17.2
8		3.4	6.2	25.3	28.1	3.0	14.5
12		3.0	6.0	27.3	38.5	2.8	14.0
	Tap.						
4		3.4	7.8	25.6	39.5	3.0	19.0
8		3.2	6.5	24.0	42.0	3.6	13.5
12		3.0	6.1	24.0	39.4	3.1	9.0

Table 13. Mean values of radioactivity in thyroids of rats exposed to 25°C[a] and 5°C

Treatment	Time (hrs) 4	8	12
	25°C		
I^{131}	2.98 (0.13)[b]	3.00 (0.39)	3.20 (0.03)
Nor.	4.02 (0.38)	1.70 (0.06)	1.18 (0.05)
Hex.	3.06 (0.42)	1.46 (0.21)	0.73 (0.40)
Tap.	2.71 (0.13)	2.72 (0.02)	2.60 (0.08)
	5°C		
I^{131}	1.30 (0.41)	1.05 (0.24)	0.59 (0.02)
Nor.	0.85 (0.10)	0.93 (0.03)	1.17 (0.06)
Hex.	1.16 (0.34)	0.61 (0.08)	0.52 (0.10)
Tap.	0.68 (0.08)	0.75 (0.16)	0.56 (0.08)

[a]Percentage of radioactivity per mg thyroid tissue.

[b]Brackets indicate standard deviation. These footnotes apply here and throughout the remainder of the tables.

Table 14. Mean values of radioactivity in thyroids of ground squirrels exposed to 25°C and 5°C

Treatment	Time (hrs) 4	8	12
	25°C		
I^{131}	0.39 (0.02)	0.39 (0.02)	0.40 (0.04)
Nor.	0.49 (0.03)	0.31 (0.01)	0.23 (0.04)
Hex.	0.50 (0.10)	0.49 (0.03)	0.38 (0.03)
Tap.	0.25 (0.01)	0.20 (0.02)	0.18 (0.02)
	5°C		
I^{131}	0.46 (0.03)	0.43 (0.06)	0.23 (0.05)
Nor.	0.19 (0.08)	0.42 (0.06)	0.47 (0.07)
Hex.	0.49 (0.04)	0.48 (0.43)	0.43 (0.05)
Tap.	0.25 (0.08)	0.25 (0.08)	0.19 (0.07)